Adult Asthma Exacerbation and Respiratory Syncytial Virus Specific IgE Detection in Sputa

Yong Won Lee

Department of Medicine

The Graduate School, Yonsei University

Adult Asthma Exacerbation and Respiratory Syncytial Virus Specific IgE Detection in Sputa

Directed by Professor Jung-Won Park

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

Yong Won Lee

December 2014

This certifies that the Doctoral Dissertation of Yong Won Lee is approved.

Thesis Supervisor : Jung-Won Park

Thesis Committee Member : In-Hong Choi

Thesis Committee Member : Tai-Soon Yong

Thesis Committee Member : Jun Chang

Thesis Committee Member : Myung Hyun Sohn

The Graduate School
Yonsei University

December 2014

ACKNOWLEDGEMENTS

Until the completion of this Doctoral Dissertation, which required long and eventful 7 years' preparations, Prof. Jung-Won Park, my thesis supervisor, led, advised, and supported me enthusiastically. I fully appreciate his instructions and feedback for my study and academic life. I'm also appreciated Prof. Chein-Soo Hong of supporting my research and life thoroughly.

The precious comments and times given by my commentators, Prof. In-Hong Choi, Tai-Soon Yong, and Myung Hyun Sohn of Yonsei University College of Medicine, and Jun Chang of Ewha Womans University College of Pharmacy have made the clarification and improvement of this study and thesis.

I'd like to thank, especially, to Dr. Joo Young Kim, Prof. Jun Chang, and their research team of Ewha Womans University College of Pharmacy, Research Institute of Pharmaceutical Sciences for their dedicated and faithful advices and supports, not confined to experiments but expanded to academic worlds.

I really thank you Jung Ho Sohn, Dr. Si-Hwan Koh, Dr. Kyung Eun Lee, and their research collegues in the Institute of Allergy (Internal Medicine and Pediatrics), and Department of Microbiology of Yonsei University College of Medicine, and research staffs in the Seoul Medical Science Institute for their countless contributions to my experiments and supports.

I also appreciate the supports of Ji-Hye Kim, Nr. Nam-Ju Kang, Dr. Hye-Jung Park, Dr. Kyung-Hee Park, Prof. Jae-Hyun Lee, and other medical staffs in the Allergy-Asthma Center of Yonsei University Health System Severance Hospital, and appreciate for backing up clinical aspects.

I would also like to thank Prof. Sung Jin Moon, Hee Jin Hwang, Sang-Yeop Yi, Hyuk-Min Lee, Hye-Yoon Kim, Jae-Wook Koh, Ki-Jun Hahn, Dong-Seok Moon, the other colleague professors and admiministra-

tors, and Dean, Prof. Min-Sung Koo in Catholic Kwandong University College of Medicine International St. Mary's Hospital for considerations and practical helps, even in critical situations.

I'm especially grateful for the endless loving care, faith, and supports of Sang-ok Lee, my mother. My parents-in-law, my brother's family and other members in my family supported me with love and faith.

Most of all, I owe a lot to my beloved wife Hana Bak, and my two daughters, Han Gyeol and Han Sol. They had helped me devotedly without uttering any complaint, and I'll never forget it.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

<ABSTRACT>

Adult Asthma Exacerbation and Respiratory Syncytial Virus Specific IgE Detection in Sputa

Yong Won Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Jung-Won Park)

Background: Acute exacerbation in asthmatics has been believed to be closely associated with respiratory viral infections. Considering the possibility of viral antigen sensitization, several studies had tried to detect respiratory syncytial virus (RSV) virus specific IgE from mice models and asthmatics. However, such approaches have not yet been fully established.

Materials & Methods: We investigated the respiratory viral detection rate, species, seasonal variation, and sequential dynamics with total 132 induced sputa samples, collected for two years, from lower airway of Korean adult asthmatics (n=60) and controls (n=10) with acutely exacerbated respiratory symptoms. Multiplex reverse trascriptase-polychain reaction (mPCR) for 12 respiratory viral species (adenovirus, rhino A virus, coronavirus OC43 and 229E/NL63, metapneumovirus, influenza A and B virus, parainfluenza viruses 1, 2, 3, and respiratory syncytial virus (RSV) A and B) were performed with those specimens. Then, we selected total 50 sputa samples and, with those specimens, detected specific IgE (in sputa supernatant) to crude RSV whole antigens and its recombinant attachment G protein core fragment (Gcf) by ImmunoCAP system (CAP), and measured Interferon-ɣ, IL-5, and IL13 by ELISA.

Results: Among 132 induced sputum samples, total 15 respiratory viral detections in mPCR were observed in asthma patients and COPD controls (n= 13, and 2, respectively). RSV was the most frequently detected

species among these (53%, 8/15). One multiple and two sequential detections were observed in three asthmatics. Among asthmatics, CAP specific IgE to Gcf (Gcf-sIgE) and IL-5 in sputa supernatant were higher in the subjects with RSV detection by mPCR ($p<0.05$, respectively). RSV-sIgE, Interferon-ɣ and IL-13 were detected in several subjects but failed to show statistical differences between the groups. In ROC analyses with the CAP measured asthmatics, the best coordinate cutoff of Gcf-sIgE, predicting RSV detection by mPCR, was 0.285 kU/L (AUC 0.73, 95% CI 0.47-0.82; SE 81.3%, SP 65.4%, PPV 59.1%, NPV 85.0%).

Conclusions: In this study, RSV was the most frequently detected viral species among the sputa from the exacerbated adult asthmatics. We applied the conventional CAP immunoassay to detecting the specific IgE antibodies to crude RSV whole antigens and its Gcf, using the sputum samples. Gcf-sIgE and IL-5 elevation in the sputa from the adult asthmatics with ever detections of RSV by mPCR, suggests that RSV protein sensitization and Th2 pathway may have roles on RSV associated asthma exacerbations in adults.

Key Words : adult asthma, exacerbation, sputum, respiratory syncytial virus (RSV), immunoglobulin E (IgE)

Adult Asthma Exacerbation and Respiratory Syncytial Virus Specific IgE Detection in Sputa

Yong Won Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Jung-Won Park)

I. INTRODUCTION

Acute exacerbation in asthmatics has been believed to be closely associated with respiratory viral infections for a long time.[1-4] For example, the relationship between asthma exacerbations and the common cold, combined with seasonal variation, has been recognized for nearly hundreds of years.[5] Such association has been well studied mainly in children, however, adult asthmatics, especially elderly, are not free from this, and a large amount of clinical and epidemiologic studies have also revealed such association.[1,5-8]

It is well known that respiratory syncytial virus (RSV) infection during early childhood can precede the subsequent airway hyperresponsiveness and the development of chronic asthma in later life.[3-7,9] Respiratory viruses including RSV and rhinovirus seem to be closely linked to exacerbations in the physiologic and immunologic intensity of an asthmatic response and can trigger 80% and 50% of childhood and adult asthma exacerbation, respectively.[3,6,7,9,10] Wos, et al. reported that human rhinovirus (HRV) was detected in the lower airway tissue of patients with asthma significantly more often than in non-asthmatic subjects, and its presence was associated with clinical features of more severe disease.[11]

Several studies reported that viral infection induced interferon prolonged

symptoms and increased severity of asthmatics.[10,12-15] Johnston, et al. suggested the role of innate immunity in pathogenesis of virus-induced asthma exacerbation.[16] They suggested that deficiency of certain interferon types in asthmatics might explain virus-induced asthma exacerbations.[15] Maybe due to such host factors, a number of respiratory viruses have been found to have latency or persistence.[17] Especially, RSV latency and persistence were reported in both mouse models and patients with chronic obstructive pulmonary disease.[18,19] Kim, et al. suggested that transition from respiratory viral infection into chronic lung disease requires persistent activation of a novel NKT cell-macrophage innate immune axis with mouse model infected by Sendai virus similar to RSV.[20] Although there are several studies contradicting above results,[21,22] dynamics of human respiratory virus and related immune cells in human airways during episodes of upper respiratory infection has been continuously evaluated.[22,23]

However, conventional respiratory viral detection methods like viral culture showed only limited clinical values because of time-cost consuming procedure and low sensitivity to fastidious viruses.[8,24] For the last one decade, polymerase chain reaction (PCR)-based methods have been considered as a candidate for the optimum method in virus detection, and several studies using PCR technique have reported that respiratory viruses are in charge of a much higher proportion of asthma exacerbations than were previously suspected.[2] For the diagnosis of acute respiratory tract infections, reverse transcriptase PCR (RT-PCR) has the advantage of detecting fastidious viral organisms such as rhinoviruses in comparison with viral culture,[12] and there have been many trials for clinical application of a multiple RT-PCR (mPCR) to specimens obtained from acute viral respiratory tract infections in children, hospitalized elderly, and exacerbated chronic obstructive lung diseases.[6-8,14,25-28]

Frequent association between respiratory viral infection and asthma exacerbation suggested the possibilities of viral sensitization among chronic asthmatics, and several studies had tried to detect virus specific immunoglobulin E (IgE) and related Th2 cytokines in these patients.[29-34]

Welliver, et al. reported RSV-specific IgE and histamines were detected in nasopharyngeal secretion from infected children.[29-31] Dakhama, et al. showed RSV-specific IgE enhanced airway responsiveness on reinfection with RSV in newborn mice model.[33,34] However, Toms, et al. reported that total IgE was low and RSV-specific IgE was undetected in sequential samples of nasopharyngeal secretion and sera of RSV infected children.[35]

Among RSV proteins, attachment protein G and fusion protein F play crucial role in infection.[36,37] Okiro, et al. tried to identify infections with RSV by using specific IgG and IgA Enzyme-Linked Immunosorbent Assays with oral-fluid samples.[38] Becker suggested that RSV G protein had highly conserved sequence containing T-cell antigenic domains which had the property of an allergen.[39,40] Such association between virus proteins and allergens might be also applied to RSV proteins, and might be applied to detect RSV-specific IgE antibody.

We hypothesized that respiratory viral infection might affect asthmatics for prolonged period and viral-specific IgE might be detected in sputum from exacerbated asthmatics with recent upper respiratory infection, especially, RSV.

So, we investigated the respiratory viral detection rate, species, seasonal variation, and sequential dynamics with induced sputa from Korean adult asthmatics with acute exacerbation and control subjects with recent upper respiratory symptoms. For these investigations, we used multiplex-PCR methods, previously reported to be superior to conventional rapid shell vial culture,[12,37] and analyzed the association between respiratory viral detection and asthma exacerbation. We also tried to apply conventional allergen-specific IgE CAP immunoassay, regarded as a quasi-standard method,[41] to detecting sputum RSV specific IgE antibody and investigated the possible sensitization of RSV proteins. Major cytokines of Th1 (interferon-gamma) and Th2 (interleukin (IL)-5 and IL-13) pathways were also measured for investigating the possible underlying mechanisms.

II. MATERIALS AND METHODS

1. Recruitment of Patients and Control Subjects

Asthmatic patients with acute exacerbation and related unscheduled visit, and non-asthmatic control subjects with recent (if possible < 21 days) upper respiratory symptoms, were recruited from the Allergy-Asthma Center of Severance Hospital (Seoul, Korea), for two years (from Dec, 2008 to Dec, 2010).

The inclusion criteria based upon the Global Initiative for Asthma (GINA) guideline[42] were as followings: 1) asthmatic symptoms such as episodic breathlessness, wheezing, cough, and chest tightness in past 12 months; 2) confirmation of the diagnosis of asthma by the measurements of lung function (spirometry or peak expiratory flow) assessing the severity of airflow limitation, its reversibility, and its variability; 3) the established asthma diagnosis by measurement of airway hyperresponsiveness for patients with symptoms consistent with asthma.

Exacerbation of asthma (asthma attacks or acute asthma) was defined in consideration of the followings: 1) episodes of progressive increase in shortness of breath, cough, wheezing, or chest tightness, or some combination of these symptoms within 48 hours; 2) increase in asthma symptoms resulting in Clinic or ER visit, and hospital admission; 3) decreases in peak expiratory flow rate (PEFR) or FEV1; 4) usage of the primary therapies for exacerbations including repetitive administration of rapid-acting inhaled bronchodilators, the early introduction of systemic glucocorticosteroids, and oxygen supplementation within 48 hours. These are also based upon the GINA guideline.[42]

With included subjects, we reviewed their medical records and analyzed the results of induced sputum analysis, total IgE, and total eosinophil counts, spirometry, bronchial reversibility test, and methacholine challenge test.

2. Sputum induction and collection

Sputum inductions were performed promptly up to 3 times, if possible on Day 0, 7, and 28 for evaluating sequential dynamics for asthmatics with mild to moderate exacerbations. Control subjects, who visited with recent respiratory symptoms, also underwent same processes. In summary, hypertonic (3%) saline was applied to subjects through an ultrasonic nebulizer (Ultra-Neb 2000, DeVilbiss-Sunrise Medical, Somerset, PA, USA) for 20 min after spirometry and bronchodilator inhalation (400 mcg of salbutamol).[43] We interrupted this process every 5 minutes for expectoration and collection of sputum and total induction time was 20 minutes or less if pulmonary function fell.[43] Several subjects with prolonged severe exacerbation, who could not underwent sputum induction procedure, expectorated sputa spontaneously. Sputum specimens underwent the processes including treatment with dithiothreitol (DTT) (Sputolysin® Reagent, EMD Millipore/Merck KGaA, Darmstadt, Germany), for further laboratory evaluations, according to the previously published protocols.[43-45]

Collected and processed sputum samples were divided into specific tubes for virus transportation and storage for later laboratory tests. Specimens were kept at 4°C, and if there any time-delay, we stored the samples at -70°C for long-term storage, before referring to the laboratories for multiplex RT-PCR, ImmunoCAP assay, and cytokine measures.

3. Multiplex reverse transcriptase-polychain reacation (mPCR)

Multiplex RT-PCR (mPCR) with induced sputum specimens were performed for 12 respiratory virus species (Adenovirus, Rhinovirus A, Coronavirus OC43, Metapneumovirus, Influenza virus A and B, Coronavirus 229E/NL63, Parainfluenza virus 1, 2, and 3, RSV A and B) by the experts in Seoul Medical Research Institutes (Seoul, Korea). This was performed according to previously described protocols in other

studies.[46,47] We performed RT-PCR using a Seeplex RV Detection Kit (Seegene Biotechnology Inc., Seoul, Korea). RNAs were extracted from 530 μL of refrigerated induced sputum samples, using a MagMax™ Viral RNA Isolation Kit (Ambion, Austin, TX, USA) with 5 μL of an internal control (SeeGene, Seoul, Korea). RNAs purified from induced sputum specimens were used for the synthesis of first-strand cDNAs by Moloney murine leukemia virus reverse transcriptase (200 U/μL; Promega, Madison, WI, USA). We used the Respiratory Virus Detection Kit-A and B (Seeplex RV detection kit, SeeGene, Seoul, Korea) for detecting eleven types of RNA and one type of DNA virus according to the manufacturer's manual. In summary, PCR was performed in a final reaction volume of 20 μL containing 6 μL of cDNA, 4 μL of 5xRV Primer, and 10 μL of 2×Master Mix.[46] The PCR protocol was 35 cycles at 94℃ for 30 sec, followed by 60℃ for 1.5 min, and 72℃ for 1.5 min, followed by a 10 min final extension at 72℃.[46] The separation of amplified PCR products were done on 2% agarose gels stained with ethidium bromide.[46] The size markers, included in each kit, had the same lengths as those of amplicons for internal control and six viruses, and if a specimen demonstrated an amplicon of the same size as one of a marker band, we scored it as positive.[46]

4. Analysis of respiratory viral detection rate, sequential dynamics, and seasonal variation in induced sputum specimens

We analyzed the viral detection rate and its sequential dynamics of mPCR among exacerbated asthmatics and non-asthmatics with respiratory symptoms. We also analyzed the monthly and seasonal variation of respiratory viral detection in subjects and compared those with the nationwide respiratory viral infection monitoring data for 2 years in Korea.

5. Comparison of clinical features between Respiratory Virus-detected Group and Non-detectors among asthmatics with exacerbations

We reviewed the medical records of asthmatics with exacerbation. We compared, if available, the clinical features including demographics, induced sputum analysis, total IgE, and total eosinophil counts, spirometry, bronchial reversibility test, and methacholine challenge test between Respiratory Virus (and/or RSV)-detected group and Non-detectors.

6. Measurement of RSV-specific IgE

A. Preparation of RSV stock

RSV stock was prepared according to protocols described in the previous study.[48] Briefly, Propagation of RSV A2 strain was done with HEp-2 cells (ATCC, Manassas, VA, USA) in DMEM (Life Technologies, Gaithersburg, MD, USA) with supplements including 3% heat-inactivated FCS, 2 mM glutamine, 20 mM HEPES, nonessential amino acids, penicillin, and streptomycin. Considering the maximal cytopathic effect of infected HEp-2 cells, viral harvesting was performed with 4~5 days' interval from cell infections. In brief, disrupting RSV infected cells by freeze-thawing (3 cycles) was followed by sonication for 1 min, and centrifugation at 300×g for 10 min, serially. The collected supernatant, combined with the cleared supernatant from the infected HEp-2 culture, was centrifuged at 75,000×g for 1 hour. The pellets, resuspended to serum-free MEM with 25-gauge needle, were underwent brief sonication. Final titers were measured by standard plaque assay.

B. Construction of RSV G protein core fragment (Gcf)

RSV G protein core fragment (Gcf) was constructed by the methods of the previous study.[48] Concisely, the amplification of coding sequence of RSV G protein spanning from amino acid residues 131 to 230 (RSV A2

strain) from cDNA was performed by PCR, and cloning into the EcoR I and Hind III sites of pET-21d (+) vector (Novagen®, EMD Millipore/Merck KGaA, Darmstadt, Germany) were done. Transforming the constructed plasmid into *E. coli* BL21 (DE3) (Novagen®, EMD Millipore/Merck KGaA, Darmstadt, Germany) was followed by inducing overexpression with isopropyl β-D-1-thiogalactopyranoside (IPTG, Takara, Shiga, Japan). Centrifugation, suspension with binding buffer (20 mM Tris, 0.5 M NaCl, pH 8.0), and sonication of bacterial cells were done. Soluble and insoluble proteins were separated by centrifugation (at 40,000×g for 20 min). Further purification were performed with the clear supernatants. Purification of expressed G protein core fragment was done using affinity chromatography with HisTrap column (GE Healthcare, Little Chalfont, UK). Then, loaded proteins, washed by binding buffer containing 20 mM imidazole, were eluted using the elution buffer (500 mM imidazole, 20 mM Tris, 0.5 M NaCl, pH 7.4). Further purification of eluted protein fractions were done with Superdex-75 columns after equilibration with PBS (GE Healthcare, Little Chalfont, UK). For endotoxin removal, the purified protein was treated with 1% Triton™ X-114 (Sigma-Aldrich Co. LLC., St. Louis, MO, USA) for 30 min at 4°C and, then, incubated at 37°C for 20 min. The phase containing endotoxin was removed using centrifugation. These processes were repeated for five cycles. For removal of residual Triton™ X-114, the protein was incubated with SM-2 beads (Bio-Rad, Hercules, CA, USA) for 2 hours at 4°C, and it was filtered through spin-X column (Costar, Washington, DC, USA). The endotoxin levels in the protein preparation were determined with the limulus amebocyte lysate (LAL) assay kit (Lonza, Switzerland) according to the manufacturer's mannual. The endotoxin level was less than 5 EU/mg. Measurement of protein concentrations was performed with Bio-Rad Protein assay (Bio-Rad Laboratories, CA, USA). Additional concentration was measured with the centrifugal filter, Amicon® ultra (Millipore, Bedford, MA, USA). For

visualization of the purification, samples were resolved on 15% SDS-PAGE and were stained using Coomassie Brilliant Blue. Western blottings for verifying the purity of Gcf were performed with RSV-specific goat polyclonal antibody (United States Biological, Salem, MA, USA) and HRP-conjugated anti-goat Ig antibody (Zymed Laboratories, CA, USA). Storage of the purified proteins was done at −80°C in aliquots until use.

C. ImmunoCAP™ specific IgE measurement (CAP assay)

ImmunoCAP™(CAP) specific IgE were measured by Phadia®250 system (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocols and the previous studies.[49,50]

Briefly, the lysates of RSV crude extracts and RSV recombinant G protein core fragments were biotinylated using the Biotin Protein Labeling Kit (Roche Diagnostics GmbH, Mannhein, Germany) according to the manufacturer's manual, and were diluted in sample diluents at 50 μL. Then, each dilution was applied to the PBS-prewashed Streptavidin ImmunoCAP™ (Thermo Fisher Scientific, Inc., Waltham, MA, USA). These CAPs were incubated for 30 minutes at room temperature and washed with PBS. Then, those CAPs were used for specific IgE measurements with the sputum supernatants by CAP assay according to the manufacturer's protocol using rabbit anti-IgE labeled with β-galactosidase and 0.01% 4-methylumbelliferyl-β-D-galactoside and detecting fluorescence by Phadia®250 system expressing results as fluorescence units (FU).[49,50] CAP assay is routinely performed in parallel with a standard curve allowing conversion from FU to IgE antibody titers in kilo international units per liter.[49] According to the previous report, one international unit of IgE is equivalent to 2.4 ng of IgE.[49] In this study, CAP assay demonstrated specific IgE titers as international unit per milliliter (IU/mL, i.e. kU/L).

7. ELISA for Interferon-gamma, IL-5, and IL-13

Total 50 sputum supernatants collected in an Eppendorf tube and centrifuged. By ELISA, Interferon-γ (BD OptEIA™ Human IFN-γ ELISA Kit II, Becton, Dickinson and Company (BD), Franklin Lakes, NJ, USA), IL-5 and IL-13 (Quantikine Human IL-5 and IL-13 ELISA kit, R&D systems, Inc., Minneapolis, MN, USA) were measured according to the manufacturers' protocols.

Briefly, 96-well plates were coated overnight with 100 μl/well of capture antibodies. Dilution of the purified anti-cytokine capture antibody to 1-4 μg/mL in binding solution were done. Then, 100 μl of diluted antibody were added to the wells of an enhanced protein-binding ELISA plate. The plates were sealed to prevent evaporation and were incubated overnight at 4°C. Blocking process was done by bring the plate to room temperature (RT), removing the capture antibody solution, and blocking non-specific binding by adding 200 μl of Blocking Buffer per well. The plates were sealed and incubated at RT for 1-2 hours. Then, washing (≥ 3 times) with PBS/Tween® was followed. The standards and samples (diluted in Blocking Buffer/Tween®) were added at 100 μl per well. Sealed plates were incubated again for 2-4 hours at RT or overnight at 4°C. Wash (≥ 4 times) with PBS/Tween® were followed. The biotinylated anti-cytokine detection antibody was diluted to 0.5-2 μg/ml in Blocking Buffer/Tween®. Adding 100 μl of diluted antibody to each well was done. The plates were sealed and incubated for 1 hour at RT. Washing with PBS/Tween® were repeated (≥ 4 times). The avidin-horseradish peroxidase (Av-HRP) conjugate (cat. no. 554058) or streptavidin-HRP (cat. no. 554066) or other enzyme conjugate were diluted to its pre-titered optimal concentration in Blocking Buffer/Tween®. Addition of 100 μl per well was done. The plates were sealed and incubate at RT for 30 min. Washing with PBS/Tween® was repeated (≥ 5 times). TMB (cat. no. 555214) according to directions or ABTS were used as a substrate. ABTS Substrate Solution was thawed within 20 min of use.

Adding 100 μl of 3% H_2O_2 per 11 ml of substrate and vortexing were done. Immediate dispensing of 100 μL into each well was followed. Incubation at RT (5-80 min) were done for color development. Finally, the optical density (OD) for each well was measured with a microplate reader (Thermo Multiskan® Ex, Vantta, Finland) set to 405 nm.

8. Statistical Analysis

Statistical analyses, data processing, and graphic works for tables and figures were performed using R version 3.1.1 (R Core Team (2014), Vienna, Austria),[51] and Microsoft® Office Excel 2007 (Microsoft Corporation, Seattle, WA, USA).

For normally distributed data, Student's t-test, Pearson's chi-square test, and Pearson's correlation test were performed, and for non-parametric analyses, Wilcoxon rank-sum test (Mann-Whitney U-test), Kruskal-Wallis test, and Spearman's correalation test were used. Receiver Operating Characteristics (ROC) curves for related statistical analyses including cutoff value, sensitivity, and negative predictive value were also be done.[41,51-53] In all cases, a two tailed p-value < 0.05 was considered to be statistically significant.

III. RESULTS

1. Demographics of enrolled subjects

Among the 139 sputum collections (Figure 1), total 132 sputum samples from 70 subjects (M/F= 27/43, Age= 52.2±15.5 year-old (18.0~77.0)) were included for mPCR during about two years (Dec 2008 ~ Dec 2010). Seven of 139 collections were excluded for incomplete process or insufficient amounts of samples. In the 70 subjects, 19 completed 2 times and 24 accomplished 3 times of sputum collection processes.

Among 70 enrolled subjects, asthmatics were 60 and control groups were 10 (chronic cough 6, bronchitis 2, and COPD 2).

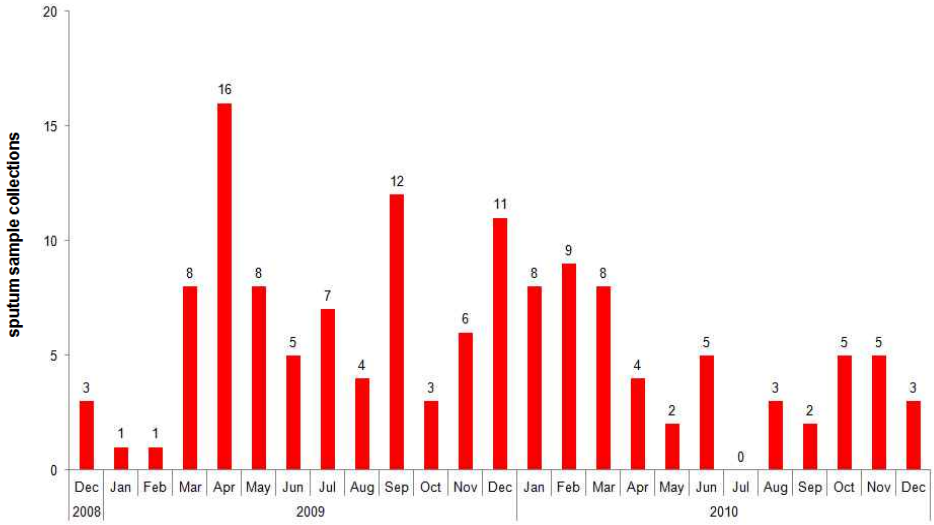

Figure 1. Time series of sputum collections (n=139).

Among the enrolled groups, 60 subjects had the available baseline results of pulmonary function test (PFT) and demonstrated 76.2±21.8% (2.1±0.960 L) of FEV1, 84.4±19.5% (2.8±1.1 L) of FVC, and 73.2±10.7% of FEV1/FVC. Sputum eosinophil fraction (n=50) were 17.3±20.7%, but the results of methacholine challenge test, and screening tests for atopy including skin prick test and phadiatop were available in only a few.

2. Respiratory viral detections in multiplex RT-PCR (mPCR)

Among 132 induced sputum samples, total 15 respiratory viral detections in mPCR were observed in asthma patients and COPD controls (n= 13, and 2, respectively).

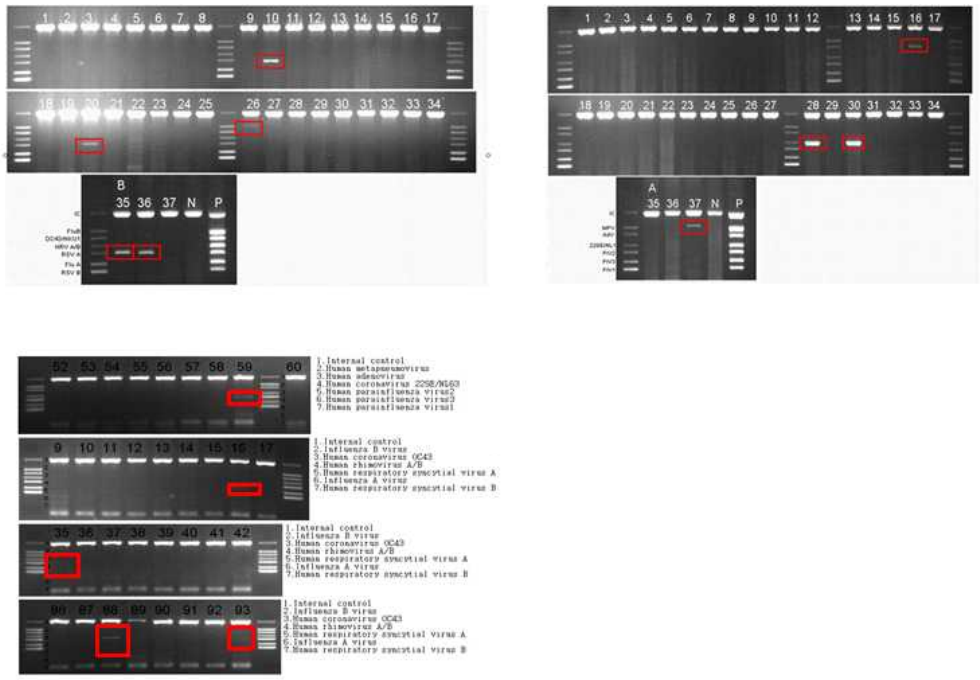

Figure 2. The results of multiplex Reverse transcriptase-Polychain Reaction (mPCR) for detection of 12 species of respiratory viruses in the sputa (n=132, partial data shown). Red rectangle: detected band.

Viral detection rates were 11% (14/132) among the sputum samples and 17% (12/70) of the subjects. RSV was the most frequently detected species among those (53%, 8/15) and the asthmatic subjects (54%, 7/13) (Figure 2 and Table 1). Seasonal variation were also observed (Table 1). For example, One multiple and two sequential detections were observed in three asthmatics (Table 1).

Table 1. Sequential viral detection by multiplex RT-PCR for 12 viral species

Subjects ID	Visit for sputum induction			Detected date
	1st	2nd	3rd	
6	**CoronaV**			April, 2009
7	**RSV A**	*Negative*	*Negative*	May, 2009
16	**RSV A**	*Negative*	*Negative*	March, 2009
17	**RSV A**	**CoronaV**		April, May, 2009
18	**RSV A**	*Negative*		May, 2009
19	*Negative*	**RSV A**	*Negative*	December, 2009
20	**RSV A, B***	*Negative*	*Negative*	December, 2009
21	*Negative*	**RSV A**	*Negative*	October, 2010
22	**ADV**	*Negative*	*Negative*	April, 2009
23	**IFV B**	**MPV**		April, May, 2009
24	**ParaIFV-2**			September, 2009
25	*Negative*	*Negative*	**IFV A**	March, 2010

*: multiple detection; blank: no visit/sputum-induction; gray zone: COPD patients (ADV: adenovirus, CoronaV: coronavirus OC43 and 229E/NL63, IFV A: influenza virus A, IFV B: influenza virus B, MPV: metapneumovirus, ParaIFV-2: parainfluenza virus 2, RSV A: respiratory syncytial virus A, RSV B: respiratory syncytial virus B)

3. Demographics of selected sputum samples for sequential dynamics, RSV and its Gcf specific IgE and cytokine measurements

Among 132 sputum samples, 50 specimens from 25 subjects were selected (Table 2) for sequential dynamics (Figure 3), RSV and its Gcf specific IgE, and cytokine measurments according to the following criteria.

1) positive viral detection in mPCR; 2) controls matching to 1) in consideration of seasonal variation and diagnosis; 3) sufficient amounts for above investigations; 4) if possible, complete initial clinically relevant data; 5) if possible, accomplished sequential collections and time intervals

The demographic data of above selected groups according to diagnoses and viral detections in mPCR were shown in Table 2.

Table 2. Demographics of selected sputum samples for sequential dynamics, RSV and its recombinant G protein core fragment (Gcf) specific IgE and cytokine measurements (n=50 samples from 25 subjects)

		Cough, n=5	COPD, n=2	Asthma, n=18		
				Virus(-), n=8	Virus(+), n=10	
					RSV(-), n=4	RSV(+), n=6
Sex(M/F)		1/4	2/0	6/2	1/3	2/4
Age(year)		41.6±15.0	55.0±2.8	48.8±18.4	62.0±4.3	57.3±11.6
BMI(kg/m^2)		22.9±2.2	21.4±0.2	24.4±2.8	24.8±2.2	28.2±2.7
PFT (base-line)	FEV1 (pred%)	2.8±0.9 L (103.0±11.8%)	1.2±0.5 L (39.1±17.7%)	2.4±0.8 L (71.6±16.9%)	1.8±0.3 L (77.7±15.3%)	1.6±0.9 L (58.7±21.9%)
	FVC (pred%)	3.3±1.0 L (103.6±9.4%)	2.1±0.7 L (54.1±20.9%)	3.3±1.0 L (82.8±15.1%)	2.4±0.3 L (86.4±13.1%)	2.2±1.1 L (69.1±19.2%)
	FEV1/ FVC	83.6±4.7%	57.0±4.3%	70.4±8.2%	73.9±6.3%	69.0±12.0%
Eosinophil (sputum)		3.8±6.9%	4.00%	13.3±10.7%	32.5±38.4%	18.3±20.5%

mean±standard deviation; pred%: percent of predicted value

Sequential dynamics showed that viral detections were only observed among subjects with chronic obstructive lung diseases (asthma and COPD) (Figure 3). RSV detections in mPCR were observed on the first visit day (5/7), and only 2 cases were detected with 7 days to 28 days of intervals from the first visit, respectively. The other viruses detected in mPCR were observed on the first day (4/7), about 1 months (2/7), and 3 months (1/7, maybe, newly infected), in turn.

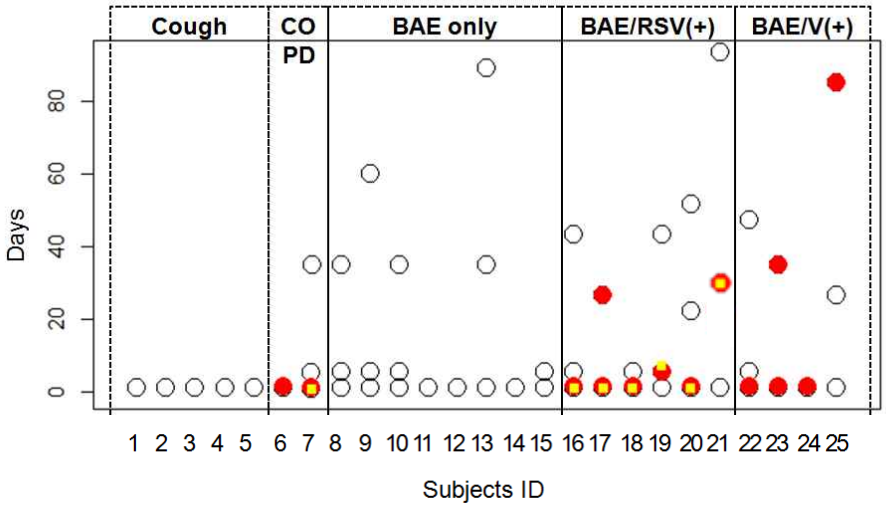

Figure 3. Sequential dynamics of respiratory viral detections in mPCR among the selected sputa (n=50) from subjects (n=25) according to sampling interval and diagnoses. Days: sampling interval, red circle: viral detected, yellow rectangle: RSV detected, BAE: bronchial asthma with exacerbation, RSV(+): RSV detected, V(+): respiratory virus detected.

4. RSV and Gcf specific IgE measurements

We applied conventional allergen-specific IgE CAP immunoassay to detecting RSV and its Gcf specific IgE antibody in sputum, and investigated the possible sensitization of RSV proteins. The whole data of these CAP specific IgE measurements were showed in Table 3.

Table 3. CAP measurement of crude RSV and its Gcf specific IgE (n=50 samples from 25 subjects; See Figure 3. legend for additional explanation.)

Subjects ID	Diagnosis/ Viral Detection	crude RSV whole antigen sIgE (CAP)		RSV Gcf sIgE (CAP)		Detected viral species (by mPCR)
		kU/L	Optical Density	kU/L	Optical Density	
1	Cough Control	*0.08*	*46*	*0.22*	*111*	*Negative*
2	Cough Control	*0.08*	*49*	*0.23*	*115*	*Negative*
3	Cough Control	*0.06*	*41*	*0.22*	*109*	*Negative*
4	Cough Control	*0.08*	*48*	*0.31*	*151*	*Negative*
5	Cough Control	*0.05*	*33*	*0.33*	*162*	*Negative*
6	COPD/V(+)	*0.06*	*41*	**0.35**	**168**	CoronaV
7	COPD/RSV(+)	*unperformed*	*unperformed*	*unperformed*	*unperformed*	RSV A*
		0.05	*34*	*0.12*	*68*	*Negative*
		0.05	*36*	*0.15*	*78*	*Negative*
8	BAE only	*0.05*	*32*	*0.18*	*93*	*Negative*
		0.05	*36*	*0.18*	*92*	*Negative*
		0.05	*35*	*0.17*	*89*	*Negative*
9	BAE only	*0.09*	*53*	*0.30*	*147*	*Negative*
		0.06	*38*	*0.19*	*97*	*Negative*
		0.08	*48*	*0.20*	*103*	*Negative*
10	BAE only	*0.08*	*49*	*0.30*	*148*	*Negative*
		0.08	*48*	*0.29*	*141*	*Negative*
		0.09	*54*	*0.28*	*138*	*Negative*
11	BAE only	*0.06*	*38*	*0.13*	*71*	*Negative*
12	BAE only	*0.07*	*45*	*0.31*	*152*	*Negative*
13	BAE only	*0.05*	*35*	*0.31*	*151*	*Negative*
		0.03	*26*	*0.24*	*119*	*Negative*
		0.05	*34*	*0.32*	*155*	*Negative*
14	BAE only	*0.06*	*38*	**0.34**	**166**	*Negative*
15	BAE only	*0.05*	*36*	*0.28*	*140*	*Negative*
		0.07	*42*	*0.26*	*130*	*Negative*
16	BAE/RSV(+)	*0.08*	*47*	*0.17*	*91*	RSV A
		0.08	*47*	*0.29*	*142*	*Negative*
		0.12	*67*	**0.35**	**168**	*Negative*
17	BAE/RSV(+)	*0.08*	*46*	*0.32*	*157*	RSV A
		0.11	*60*	*0.30*	*149*	Corona
18	BAE/RSV(+)	*0.06*	*39*	*0.12*	*68*	RSV A
		0.05	*36*	*0.13*	*70*	*Negative*
19	BAE/RSV(+)	*0.08*	*47*	*0.33*	*161*	*Negative*
		0.04	*30*	**0.34**	**166**	RSV A
		0.06	*37*	*0.32*	*158*	*Negative*
20	BAE/RSV(+)	*0.05*	*34*	*0.29*	*142*	RSV A,B
		0.05	*35*	*0.30*	*146*	*Negative*
		0.05	*34*	*0.29*	*142*	*Negative*
21	BAE/RSV(+)	**0.52**	**244**	**0.52**	**245**	RSV A
		0.07	42	**0.36**	**174**	*Negative*
		0.04	31	**0.35**	**169**	*Negative*
22	BAE/V(+)	0.06	40	0.28	139	ADV
		0.06	39	0.25	125	*Negative*
		0.08	46	0.26	128	*Negative*
23	BAE/V(+)	0.06	38	0.21	106	IFV B
		0.08	46	0.27	133	MPV
24	BAE/V(+)	0.04	29	0.12	64	ParaIFV2
25	BAE/V(+)	0.05	33	0.29	144	*Negative*
		0.05	33	0.29	144	*Negative*
		0.04	29	0.28	139	IFV A

Crude RSV specific IgE were detected in several subjects but failed to show statistical differences between the subject groups. Among asthmatics, CAP specific IgE to Gcf (Gcf-sIgE) in sputa supernatant was higher in the subjects with RSV detection by mPCR (p=0.012) (Figure 4).

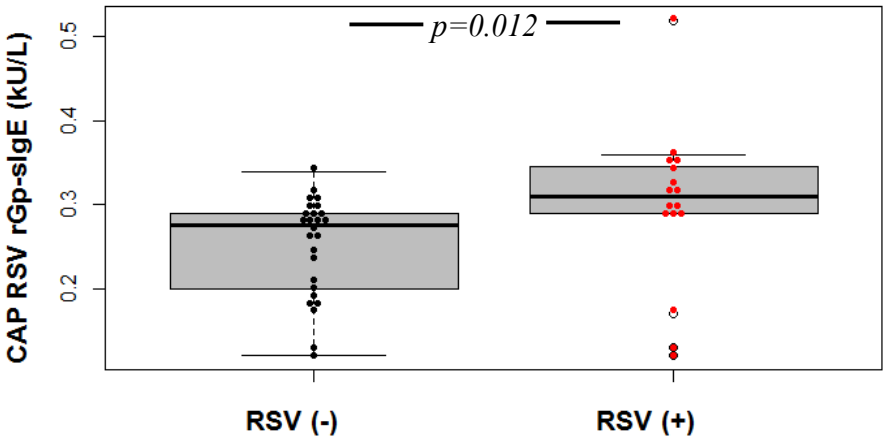

Figure 4. Comparison of CAP RSV recombinant G protein core fragment specific IgE (Gcf-sIgE) measurement among the exacerbated adult asthmatics between the RSV undetected (-) and ever detected (+) groups.

5. Cytokine measurements (Interferon-gamma, IL-5, and IL-13)

Major Th1 (interferon-gamma) and Th2 (IL-5 and IL-13) cytokines were measured with the sputum supernatants from the subjects for investigating possible underlying mechanisms. Interferon-gamma was detected in the several subjects but failed to show statistical differences between the subject groups (Figure 5A). Among the exacerbated adult asthmatics, IL-5 in sputa supernatant was higher in the subjects with RSV detection by mPCR ($p=0.013$) (Figure 5B). However, IL-13 was detected in only one control subject (data not shown) and could not be statistically analysed.

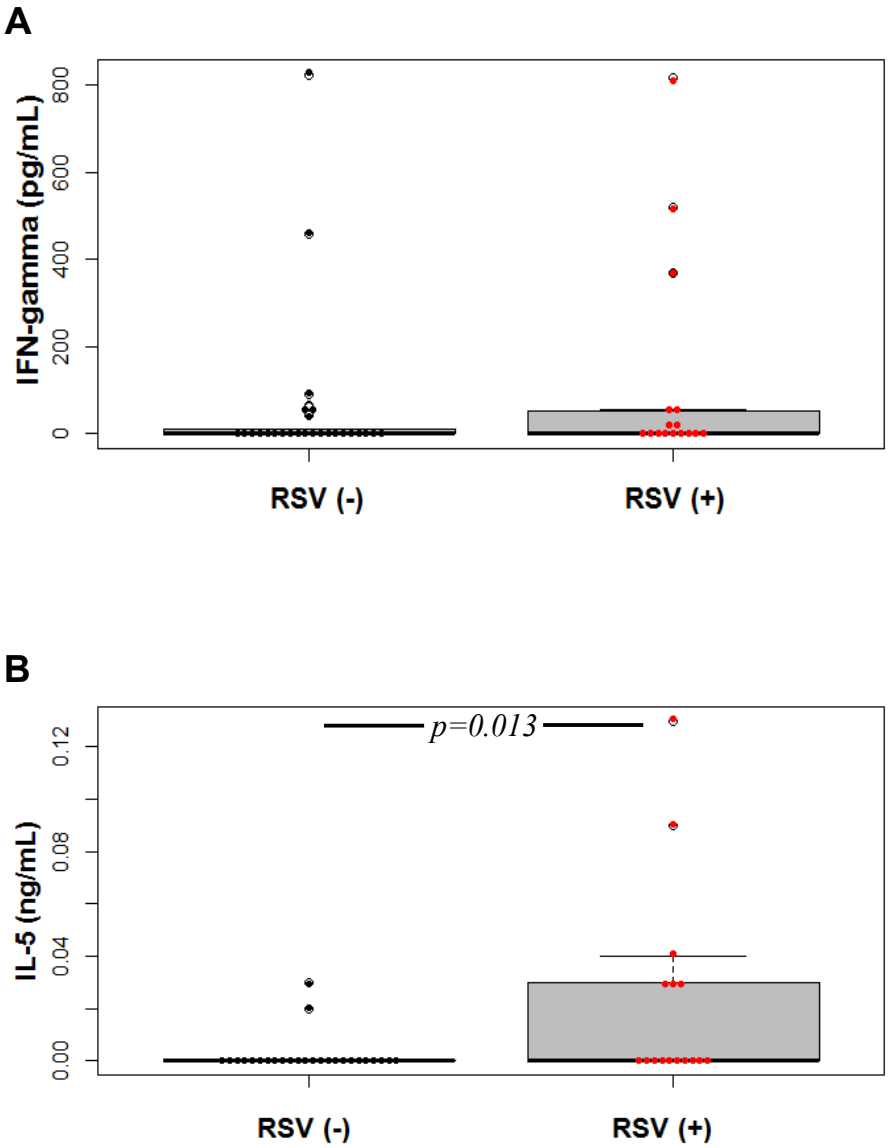

Figure 5. Comparisons of Interferon (IFN)-gamma (A) and IL-5 (B) measurements among the exacerbated adult asthmatics between the RSV undetected (-) and ever detected (+) groups.

6. Receiver operating characteristic (ROC) curve analysis for CAP RSV Gcf-sIgE measured asthmatics with exacerbation

In the ROC analyses, CAP RSV Gcf-sIgE for the exacerbated asthmatics with or without ever detection of RSV by mPCR showed the fair accuracy of 0.73 measured by the area under the ROC curve (AUC) (95% confidence interval (95% CI) 0.47-0.82; sensitivity (SE) 81.3%, specificity (SP) 65.4%, positive predictive value (PPV) 59.1%, negative predictive value (NPV) 85.0%), and demonstrated the best coordinate cutoff value (the highest value that sensitivity and specificity can be jointly attained) at 0.285 kU/L (Figure 7).[41,51-53]

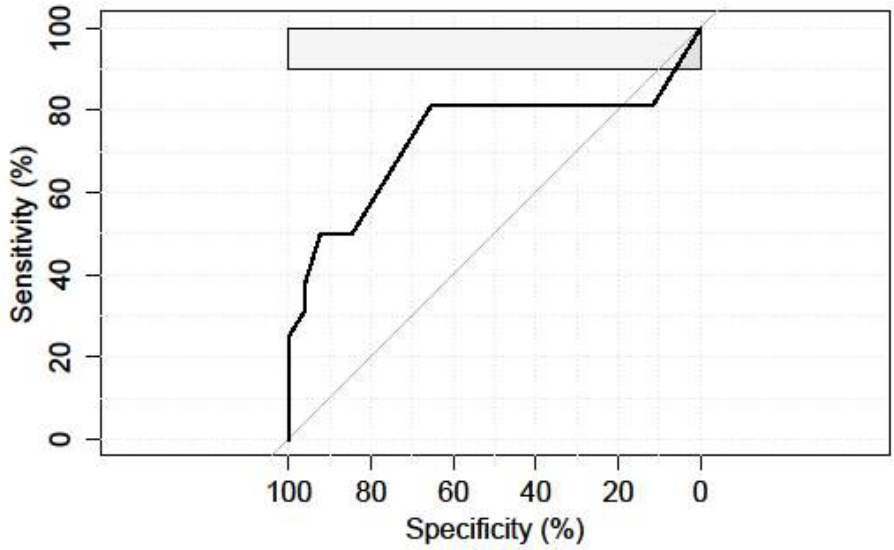

Figure 6. Receiver operating characteristic (ROC) curve analysis of CAP RSV Gcf-sIgE for the exacerbated asthmatics with or without ever detection of RSV by mPCR (area under curve (AUC) 0.73, 95% confidence Interval 0.47-0.82; sensitivity 81.3%, specificity 65.4%, positive predictive value 59.1%, negative predictive value 85.0%; The best coordinate cutoff value of RSV Gcf-sIgE discriminating the exacerbated asthmatics with or without ever detection of RSV by mPCR was 0.285 kU/L).

IV. DISCUSSION

In this study, RSV was the most commonly detected species by mPCR for 12 respiratory viral species, using sputum samples induced from lower airways of adult asthmatics with acute exacerbation and control subjects with upper respiratory symptoms. In the subgroup analysis, RSV was also the most frequently detected viral species among acutely exacerbated asthmatics. Conventional allergen-specific IgE CAP immunoassay can be applied to detect crude RSV and its recombinant G protein core fragment specific IgE antibodies in sputum specimens. Among the asthmatics with acute exacerbation, RSV ever-detected (by mPCR) subjects showed higher Gcf-sIgE and IL-5 levels than the RSV-undetected group. These results suggest clinical clues for the mechanism of respiratory viral infection induced acute exacerbations in adult asthmatics, which has not yet been fully evaluated.

The overall respiratory viral detection rate of this study was 11.4% among the 132 induced sputum samples (118 from asthmatics and 14 from control subjects with recent upper respiratory symptoms). This detection rate seems to be lower than the previous studies using PCR techniques.[8,54-56] Early studies had reported low respiratory viral detection rates, for they used virus detection method with low sensitivity for rhinoviruses and corona viruses, causing the majority of colds.[2] For example, Beasly, et al. reported only 10% of viral identification rate among 178 asthma exacerbations.[57]

Later, the role of viruses in asthma exacerbations were investigated by multiplex PCR technique in several studies,[54-56] and then more rapid multiplex PCR was developed.[24] With nasopharyngeal swab samples from elderly subjects, Falsey, et al. reported higher RSV detection rates of RSV strain-specific RT-PCR assay and serologic enzyme immunoassay using IgG responses to purified RSV glycoproteins than those of viral

culture methods.[8] The previously reported viral detection rate in respiratory specimens from children with asthma exacerbation ranged from 38% to 63.1%,[55,56] but control group showed 23.4% of viral detection rate.[56] In adult patients, acute asthma showed 41% of viral detection rate and near-fatal asthma demonstrated 59% of viral detection rate.[54]

Nationwide respiratory viral monitoring data of the overlapping periods with this study has been opened to public by the Korea Influenza and Respiratory Surveillance System (KINRESS) and the Surveillance network for severe Lower Respiratory tract Infection in infants and children (SLRI) sponsored by Korea Center for Disease Control and Prevention (KCDC).[58,59] This surveillance had been performed by mPCR/RT-PCR for 9 respiratory viral species (Influenza virus, Adenovirus, Parainfluenza virus, RSV, Enterovirus, Bocavirus, Coronavirus, Rhinovirus, and Metapneumovirus) using throat swab from adult outpatients visited with common cold symptoms, and nasopharyngeal aspirates from children (under 5 years of age) admitted with respiratory infections.[58] The KINRESS and the SLRI reported 52.9% and 73.7% of viral detections rates in 2010, respectively.[58]

The relatively low viral detection rate of this study may be caused by differences in collection sites (upper vs. lower respiratory tract) and process of specimens (dilution factor, RT-PCR methods, etc.), and clinical characteristics of subjects groups (general population vs. mainly, asthmatics). It should be considered that this study was performed with outpatients who could undergo sputum induction procedures collecting mainly lower airway specimens.

The results in this study showed seasonal variations of respiratory viral detection frequency and species in sputum samples from acutely exacerbated asthmatics and control subjects. The respiratory detections including RSV showed two peaks in the seasons of spring (March to May) and autumn (September to December), and these findings corresponded to the previously mentioned KCDC surveillance reports

during the periods overlapped to this study.[58,59]

Among the sputum samples from the asthmatics with acute exacerbation, RSV detection rate was 5.9% (7/118), and RSV was the most frequently found viral species in this study. According to the nationwide KCDC/ KINRESS surveillance reports from 2010 to 2012, RSV showed average 4.5% of detection rate, and ranked only the 4th frequently detected viral species next to human rhinovirus (15.4%), influenza virus (14.0%), and adenovirus (8.3%) among general population with respiratory symptoms.[59] The differences of viral detection rates between this study and nationwide surveillance enrolled general subjects suggests that RSV infection may be more closely associated with acute asthma exacerbation than the other respiratory viral species.

RSV had not been recognized as a potentially serious problem in older adults until the 1970s, however, RSV outbreaks in long-term care facilities arouse public opinion that the RSV might be an important cause of illness in community-dwelling elderly.[60-62] There had been several reports that RSV accounted for deaths in elderly, and it was assumed that approximately 10,000 deaths annually in elderly (over the age of 65 years) were associated with RSV infections in the United States.[8,63-67] Two meta-analyses showed that RSV has caused more than 3.4 million episodes of acute lower respiratory infections in 2005, while seasonal influenza caused more than 20 million episodes of such infections worldwide in 2008.[68,69] Gaut ER, et al. calculated disease burden of the most commonly detected viruses among hospitalized patients using the disability adjusted life year model, which the World Health Organization endorsed.[70] The calculation showed strikingly different disease burden profiles in children and adults, for example, adenovirus was among the leading causes of respiratory presentation in children but not adults.[70] However, RSV and influenza A were consistently observed as one of the greatest causes of disease regardless of sampled population.[70]

Lysholm, et al. tried to characterize the viral microbiome by metagenomic sequencing using nasopharyngeal aspirates from the patients with severe lower respiratory tract infections, and reported that Paramyxoviridae (RSV, metapneumovirus, and parainfluenza virus), Picornaviridae (rhinovirus), and Orthomyxoviridae (influenza virus) were the 3 most common causes for severe lower respiratory tract infections in children, and some adults.[71] Among those, the most abundant viral family was Paramyxoviridae (38%: RSV 80%, metapneumovirus 15%, and parainfluenza virus 4%), which outnumbered Picornaviridae (31%: rhinovirus A 65%, B 0.1%, and C 35%).[71] It was also reported that rhinoviruses were one of significant pathogens in all groups except those aged 16-64 years.[70]

Considering these previous studies, no detection of rhinovirus species in this study may be partially caused by the differences in ages of subjects, type and collecting sites of specimen, morbid diseases, and limited mPCR detection confined to rhinovirus A, but it still remains to be more elucidated.

We postulated that respiratory viral infection might affect asthmatics for prolonged period, however, this study failed to show any prominent and prolonged sequential dynamics of respiratory viral detections. It is still controversial whether respiratory viruses are simple exacerbaters or potent causes with latency and persistence of asthma. Several studies reported that respiratory viruses, especially RSV, showed such latency and persistence in chronic obstructive lung disease including asthma, even despite T cell immunity.[25,26] However, Wood, et al. suggested that persistent airway obstruction after respiratory viral infection was not associated with airway inflammation,[21] and van Elden, et al. suggested that enhanced severity of virus associated lower respiratory tract disease in asthmatics might not be associated with delayed clearance (i.e. persistence) and increased load of respiratory viruses in the upper airway.[22]

The demographic data of selected groups according to diagnoses and viral detections in mPCR (Table 2) showed several interesting findings.

Although these findings were not statistically significant due to the limited sample size (50 samples from 25 subjects), the subgroup of the RSV detected asthmatics showed a trend of higher body mass index (BMI) than the RSV undetected asthmatics or control subjects (p=0.07). The RSV detected asthmatics also tended to have lower baseline FVC and FVC % predicted value than the RSV undetected asthmatics or the control subjects (p=0.07 and 0.13, respectively). These findings might suggested that acute exacerbations in the asthmatics with high BMI and low baseline lung function tend to be associated with RSV infections.

Several previous studies have suggested that increased body weight is associated with changes in certain immune functions, and less immune response to vaccines,[72] and obese patients frequently experience worse outcomes relating to infectious diseases than the thinner counterparts.[73] Zimmerman, et al. reported that higher BMI was associated with respiratory influenza infection, though such finding was not prominent in RSV infection.[74] Furthermore, decreased lung function may be associated with prior events of a childhood RSV infection and its sequela.[9,75] Recently, Backman, et al. carried out a 30-year follow-up on 43 adults admitted for RSV lower respiratory tract infections (27 for bronchiolitis and 16 for pneumonia), and such hospitalization in infancy was related to an increased risk of permanent obstructive reduction of lung function in adulthood.[76,77] They also reported higher asthma risk after hospitalization for bronchiolitis.[76,77]

To find the targets of viral infection to a host-protective mechanism in early life, which increases susceptibility to allergic disease, Krishnamoorthy, et al. has suggested that early infection with RSV impairs regulatory T cell function and increases susceptibility to allergic asthma with infant mouse models.[78] In repeated infection of infant mice, they observed that RSV induced a Th2-like effector phenotype in Treg cells and attenuated tolerance to an unrelated antigen (i.e. allergen) by promoting a Th2-type inflammatory response in the lung.[78]

Does RSV protein specific IgE exist, like allergens specific IgE? As previously mentioned, there have already been several investigations prove the existence of RSV-specific IgE existence using ELISA methods with in nasopharyngeal secretion, and sera from infected children and mice models.[29,30,31,33,34] However, to our knowledge, this study is the first application of conventional ImmunoCAP system to detect RSV and its Gcf specific IgE antibodies, using sputum samples. As previously mentioned, this system is clinically available and established as a quasi-standard in immunoassays for serum allergen-specific IgE.[41] By this approach, we tried to demonstrate the possible sensitization of RSV proteins, like inhalant allergens. As a result, we could detect RSV and its Gcf specific IgE antibodies in sputum samples from the subjects. One of them with RSV detection by mPCR, showed even higher IgE level (0.52 kU/L) than conventional lower cutoff value for serum samples (0.35 kU/L).[49,50]

Does these findings have any clinical meaning and reflect the need of real clinical situations? Though there has been previously mentioned controversies in the existence of RSV-specific IgE detection in nasopharyngeal secretion from the RSV infected subjects,[35] our study confirmed the existence by clinically available and standardized specific IgE detecting immunoassay system. Furthermore, recombinant RSV G protein core fragments may be used better in the measurement of RSV-specific IgE than total crude RSV antigens, because recombinant protein fragments can be constructed by engineered technique which is more suitable for industrial productions. Such future possibilities of clinical application will be more discussed in the latter part.

Only a few of asthmatics among RSV detected by mPCR, showed higher than conventional cutoff range for serum samples. But the asthmatics with ever detection of RSV by mPCR, showed higher RSV recombinant G protein core fragment specific IgE and IL-5 levels than RSV undetected asthmatics. It can be assumed that the possible sensitizer for some of acutely exacerbated asthmatics is more likely the comprising

protein like G protein than crude RSV whole antigens. Such component resolved diagnostic approach using the recombinant allergens has been common in allergen investigations and has been strengthened by molecular and microarray technique,[41,79-81] and can be applied to investigation of viral protein sensitization and its impact on asthma exacerbation.

This study has several strengths and limitations as followings.

This investigation was performed with adult asthmatic exacerbations and their induced sputum specimens from lower airways rather than nasopharyngeal aspirates. This is an outpatient-based study reflecting real clinical situations. Although there have been several Korean domestic studies using PCR technique for respiratory viral detection with sputum samples from upper and lower airways, most of those are limited to children,[46,47,82] or small sized adult groups mixed asthmatics with other chronic obstructive lung disease patients.[83] Therefore, the results of this study can suggest more clinical clues for the association of respiratory viral infections with adult asthma exacerbation.

However, limited sample size from single asthma-allergy center of single institution and no inclusion of healthy controls are the weak points of this study. Considering the limited outpatient based study settings, it was inevitable and could be the possible cause of selection bias, that this study enrolled mainly asthmatic subjects with acute exacerbations and only small sized controls with recent respiratory symptoms. This weakness was accompanied by frequent loss to follow up for serial viral dynamic observations, and resulted in the limited available clinical data.

Because of the difficulties in sputum induction procedures, the collected amount of some sputum samples were not enough. This shortage of sputum amounts caused the limitations in measuring enough times and types of IgE and cytokine, as the prior respiratory mPCR requiring considerable amounts of specimens.

As previously discussed, respiratory detections showed seasonal variations

and those were also overlapped with the inhalant allergen peak seasons. So it is still undistinguishable whether respiratory viruses are direct cause, simple trigger assisting allergens, or even just bystander of allergic inflammations. This point is the insuperable limitation and discussion points of this study. However, Cheung, et al. claimed that there had been several evidences for a post-viral "atopic cycle" that could explain the development of multiple allergen sensitization, and supported the "viral hypothesis", and, in particular, the role of RNA viruses, including RSV, in the development of atopic diseases.[84]

Busse, et al. suggested that deficiencies in antiviral activity and the integrity of the airway epithelial barrier could make asthmatics prone to have severe respiratory viral infections of the lower airway, increasing the risk of exacerbation.[85] We can also consider the possibility of cross-reactions between viral antigens and inhalant allergens, like in the pollen cross-allergenicity.[86] Interestingly, Becker suggested that gp41 heptad repeat 2 (HR2) possesses an amino acid domain that resembles the allergen domain in *Aspergillus fumigatus Asp f 1* protein.[87]

Although, to our knowledge, this study is the first trial to measure the specific IgE to crude RSV whole antigens and its recombinant G proteins core fragments by conventional ImmunoCAP system, there were several limitations due to the unestablished conditions of this original approach. We tried to performed the CAP assay according to the manufacturer's manual and the previously reported studies using serum samples.[49,50] Although there were several proposals for measurement of total and specific IgE in sputum,[88-90] sputum processing for ImmunoCAP system is still not fully established. So there must be limitations related to conditioning, calibration, unestablished standard and reference range, titration and quantification, sputum processing and dilution effects. For example, it had been suggested that sputum processing with too high concentration of dithiothreitol (DTT) might reduce the disulfide bonds in the IgE-molecule.[88-90] These limitations should be considered in mPCR

detection performance, either.

But our study also suggests some clinical clues, like a possible cutoff value of RSV Gcf-sIgE for discriminating the exacerbated asthmatics with or without ever detection of RSV by mPCR, suggesting the possible RSV infection experience. The ROC analyses for CAP sputum RSV Gcf-sIgE of the exacerbated adult asthmatics, showed fair accuracy (AUC 0.73) and demonstrated good sensitivity (81.3%) and negative predictive value (NPV) (85.0%) at the best coordinate cutoff value (0.285 kU/L).[51-53] These results might suggest that RSV Gcf-sIgE could be the fair to good screening tool to identify the RSV associated asthma exacerbation, that might have different and more severe clinical features than the others.

There are another limitations in this investigation. This study was performed with only sputum specimens, serum samples as powerful references were unable to draw from subjects. Other major serologic parameters of conventional studies for virology, like IgG, IgG4, and IgA, were unmeasured. These should have been performed like the previous report.[91] However, Khan, et al. asserted in their review with a total of 1,643 publications that only several studies focused on IgE and the viral risk of asthma, and they emphasized the development of future therapies for post-viral atopic disease related to antiviral IgE.[92]

Recently, the existence and importance of local IgE in nasal mucosa of patients with allergic rhinitis, very closely associated disease entitity with asthma, have been suggested.[93,94] Baba, et al. reported local class switching to IgE, production of IgE and IgE localization to the surface of mast cells in eosinophilic chronic rhinosinusitis among the Japanese population.[93] Meng, et al. suggested that immunoglobulin free light chain might play an important role in inducing inflammation of local nasal mucosa in the patients with allergic rhinitis and nonallergic rhinitis.[94] Taken together, IgE detection in sputum samples locally collected from lower airways may have clinical significance, to the certain extent, even without serologic evaluation.

Limited cytokine measurement is also a critical weakness of this study. We investigated only Interferon-gamma for Th1 pathway, and IL-5 and IL-13 for Th2 pathway, considering shortage of sputum samples and very low detection rates, as in IL-4 mesurement. However, there are several cytokines crucial for investions of mechanisms for asthma, such as IL-2, IL-4 (especially involved in IgE class switch), IL-10, IL-17, IL-22, and etc.

Recently, Ghebre, et al. performed factor and cluster analyses on 18 sputum cytokines including the above mentioned, with severe asthma and moderate-to-severe chronic obstructive pulmonary disease (COPD) patients.[95] They suggested that sputum cytokine profiling could determine distinct and overlapping groups of asthmatics and COPD patients, supporting both the British and Dutch hypotheses.[95] Bafadhel, et al. had also studied profiling of sputum inflammatory mediators in asthma and COPD, and reported that recovery of sputum mediators sensitive to dithiothreitol (DTT) could be improved using the sputum processing technique, which they described.[96] These approach and results can be applied to future researches related to this study.

Nevertheless, Rothers, et al. also reported that productions of IL-5, and IL-13, Interferon-gamma, measured in this study, and IL-4 were also associated with total IgE levels in active asthma through age 5 years, although relations varied temporally.[97] Furthermore, Crestani, et al. showed that mitogen-stimulated IL-5 production was associated with *in vivo* total IgE levels, independent of other cytokines and circulating eosinophils.[98]

Our research suggests that respiratory viral proteins including crude RSV whole antigens, and its recombinant component protein fragments including G protein core fragments, can be possible candidates for specific IgE measurements by conventional immunoassay system using non-invasive sputum inductions from asthmatics with acute exacerbation. Such approach can be used for various clinical applications, including mechanism investigation (possible allergenicity of viral proteins, etc.),

prediction, diagnosis, treatment and vaccine development[99] for respiratory virus associated with asthma exacerbation in near future.

For example, this approach may be used as a diagnostic tool for identifying RSV infection related asthma exacerbations, based upon clinical validation by further investigations, like the other recent diagnostic approaches.[100] Furthermore, there are various specific IgE measuring systems, which clinically available and standardized for such applications.[41,101] This approach might be used for the pretests for determinating the indication of anti-IgE therapy, which has been used for severe asthma cases. Considering the high cost of the anti-IgE drug (omalizumab), our study can provide clinical clues for future researches to identify biomarkers predicting responses to anti-IgE therapy.[102,103]

Further large scaled and more detailed clinical investigations will be helpful for development and expansion of diagnosis and treatment tools for respiratory virus related asthma exacerbations.

V. CONCLUSION

In this study, RSV was the most frequently detected viral species among the sputa from the exacerbated adult asthmatics. We applied the conventional CAP immunoassay to detecting the specific IgE antibodies to crude RSV whole antigens and its Gcf, using the sputum samples. Gcf-sIgE and IL-5 elevation in the sputa from the adult asthmatics with ever detections of RSV by mPCR, suggests that RSV protein sensitization and Th2 pathway might have roles on RSV associated asthma exacerbations in adults.

REFERENCES

1. Busse WW. The contribution of viral respiratory infections to the pathogenesis of airway hyperreactivity. Chest 1988; 93: 1076-82.
2. Mallia P, Johnston SL. How viral infections cause exacerbation of airway diseases. Chest 2006; 130: 1203-10.
3. Schaller M, Hogaboam CM, Lukacs N, Kunkel SL. Respiratory viral infections drive chemokine expression and exacerbate the asthmatic response. J Allergy Clin Immunol 2006; 118: 295-302.
4. Friedlander SL, Busse WW. The role of rhinovirus in asthma exacerbations. J Allergy Clin Immunol 2005; 116: 267-73.
5. Johnston NW. The similarities and differences of epidemic cycles of chronic obstructive pulmonary disease and asthma exacerbations. Proc Am Thorac Soc 2007; 4: 591-6.
6. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ 1993; 307: 982-6.
7. Seemungal T, Harper-Owen R, Bhowmik A, Moric I, Sanderson G, Message S, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001; 164: 1618-23.
8. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med 2005; 352(17): 1749-59.
9. Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt Susanne, Sigurbergsson F, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med 2005; 171: 137-141.
10. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. BMJ 1995; 310: 1225-9.
11. Wos M, Sanak M, Soja J, Olechnowicz H, Busse WW, Szczeklik A. The presence of rhinovirus in lower airways of patients with bronchial asthma.

Am J Respir Crit Care Med 2008; 177: 1082-9.
12. Johnston SL, Sanderson G, Pattemore PK, Smith S, Bardin PG, Bruce CB, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. J Clin Microbiol. 1993; 31:111-7.
13. Openshaw PJ, Tregoning JS. Immune responses and disease enhancement during respiratory syncytial virus infection. Clin Microbiol Rev 2005; 18(3): 541−55.
14. Mallia P, Message SD, Kebadze T, Parker HL, Kon OM, Johnston SL. An experimental model of rhinovirus induced chronic obstructive pulmonary disease exacerbations: a pilot study. Respir Res. 2006; 7: 116.
15. Edwards MR, Johnston SL. Deficient interferon in virus-induced asthma exacerbations. Clin Exp Allergy 2008; 38: 1416-8.
16. Johnston SL. Innate immunity in the pathogenesis of virus-induced asthma excerbations. Proc Am Thorac Soc 2007; 4: 267-70.
17. Tauro S, Su Y-C, Thomas S, Schwarze J, Matthaei KI, Townsend D, et al. Molecular and cellular mechanisms in the viral exacerbation of asthma. Microbes and Infection 2008; 10: 1014-23.
18. Schwarze J, O'Donnell DR, Rohwedder A, Openshaw PJ. Latency and persistence of respiratory syncytial virus despite T cell immunity. Am J Respir Crit Care Med 2004; 169: 801-5.
19. Sikkel MB, Quint JK, Mallia P, Wedzicha JA, Johnston SL. Respiratory syncytial virus persistence in chronic obstructive pulmonary disease. Pediatr Infect Dis J 2008; 27: S63-70.
20. Kim EY, Battalile JT, Anand PC, You Y, Agapov E, Grayson MH, et al. Persistent activation of an innate immune axis translates respiratory viral infection into chronic lung disease. Nat Med 2008; 14: 633-40.
21. Wood LG, Powell H, Grissell T, Nguyen TTD, Shafren D, Hensley M, et al. Persistent airway obstruction after virus infection is not associated with airway inflammation. Chest 2007; 131: 415-23.
22. van Elden LJR, Sachs APE, van Loon AM, Haarman M, van de Vijver

DA, Kimman TG, et al. Enhanced severity of virus associated lower respiratory tract disease in asthma patients may not be associated with delayed viral clearance and increased viral load in the upper respiratory tract. J Clin Virol 2008; 41: 116-21.

23. Heidema J, Rossen JWA, Lukens MV, Ketel MS, Scheltens E, Kranendonk MEG, et al. Dynamics of human respiratory virus-specific CD8+ T cell responses in blood and airways during episodes of common cold. J Immunol 2008; 181: 5551-9.
24. Lam WY, Yeung AC, Tang JW, Ip M, Chan EW, Hui M, et al. Rapid multiplex nested PCR for detection of respiratory viruses. J Clin Microbiol 2007; 45: 3631-40.
25. Gilbert LL, Dakhama A, Bone BM, Thomas EE, Hegele RG. Diagnosis of viral respiratory tract infections in children by using a reverse transcription-PCR panel. J Clin Microbiol 1996; 34: 140-3.
26. Ellis JS, Fleming DM, Zambon MC. Multiplex reverse transcription-PCR for surveillance of influenza A and B viruses in England and Wales in 1995 and 1996. J Clin Microbiol 1997; 35: 2076-82.
27. Gruteke P, Glas AS, Dierdorp M, Vreede WB, Pilon JW, Bruisten SM. Practical implementation of a multiplex PCR for acute respiratory tract infections in children. J Clin Microbiol 2004; 42: 5596-603.
28. Freymuth F, Vabret A, Cuvillon-Nimal D, Simon S, Dina J, Legrand L, et al. Comparison of multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to hospital with an acute respiratory illness. J Med Virol 2006; 78: 1498-504.
29. Welliver RC, Wong DT, Sun M, Middleton E, Vaughan RS, Ogra PL. The development of RSV-specific IgE and the release of histamine in nasopharyngeal secretions after infection. N Engl J Med 1981; 305: 841−6.
30. Welliver RC, Sun M, Rinaldo D, OgraPL. Predictive value of respiratory syncytial virus-specific IgE responses for recurrent wheezing following bronchiolitis. J Pediatr 1986; 109: 776-80.
31. Welliver RC, Duffy L. The relationship of RSV-specific immunoglobulin

E antibody responses in infancy, recurrent wheezing and pulmonary function at age 7–8 years. Pediatr Pulmonol 1993; 15: 19–27.
32. Roman M, Calhoun WJ, Hinton KL, Avendano LF, Simon V, Esobar AM. Respiratory syncitial virus infection in infants is associated with predominant Th-2-like response. Am J Respir Crit Care Med 1997; 156: 190-5.
33. Dakhama A, Park JW, Taube C, Chayama K, Balhorn A, Joetham A, et al. The role of virus-specific immunoglobulin E in airway hyperresponsiveness. Am J Respir Crit Care Med 2004; 170: 952-9.
34. Dakhama A, Lee YM, Ohnishi H, Jing X, Balhorn A, Tkeda K, et al. Virus-specific IgE enhances airway responsiveness on reinfection with respiratory syncytial virus in newborn mice. J Allergy Clin Immunol 2009; 123: 138-45.
35. Toms GL, Quinn R, Robinson JW. Undetectable IgE responses after respiratory syncytial virus infection. Arch Dis Child 1996; 74: 126-30.
36. Fleming E, Kolokoltsov AA, Davey RA, Nichols JE, Robers Jr. NJ. Respiratory syncytial virus F envelope protein associates with lipid rafts without a requirement for other virus proteins. J Virol 2006; 80: 12160-70.
37. Yu JR, Kim S, Lee JB, Chang J. Single intranasal immunization with recombinant adenovirus-based vaccine induces protective immunity against respiratory syncytial virus infection. J Virol 2008; 82: 2350-7.
38. Okiro EA, Sande C, Mutunga M, Medley GF, Cane PA, Nokes DJ. Identifying infections with respiratory syncytial virus by using specific immunoglobulin G (IgG) and IgA enzyme-linked immunosorbent assays with oral-fluid samples. J Clin Microbiol 2008; 46: 1659-62.
39. Becker Y. Respiratory syncytial virus (RSV) evades the human adaptive immune system by skewing the Th1/Th2 cytokine balance toward increased levels of Th2 cytokines and IgE, markers of allergy − a review. Virus Genes 2006; 33: 235-52.
40. Becker Y. Respiratory syncytial virus (RSV)-induced allergy may be controlled by IL-4 and CX3C fractalkine antagonists and CpG ODN as

adjuvant: hypothesis and implications for treatment. Virus Genes 2006; 33: 253-64.
41. Lee YW, Sohn JH, Lee JH, Hong CS, Park JW. Allergen-specific IgE measurement with the IMMULITE 2000 system: Intermethod comparison of detection performance for allergen-specific IgE antibodies from Korean allergic patients. Clinica Chimica Acta 2009; 401: 25–32.
42. From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2007 and 2010. Available from: http://www.ginasthma.org/.
43. Scheicher ME, Terra Filho J, Vianna EO. Sputum induction: review of literature and proposal for a protocol. Sao Paulo Med J 2003; 121: 213-9.
44. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med 1996; 154: 308-17.
45. Bacci E, Cianchetti S, Carnevali S, Bartoli ML, Dente FL, Di Franco A, et al. Induced sputum is a reproducible method to assess airway inflammation in asthma. Mediators Inflamm 2002; 11: 293-8.
46. Yoo SJ, Kuak EY, Shin BM. Detection of 12 respiratory viruses with two-set multiplex reverse transcriptase-PCR assay using a dual priming oligonucleotide system. Korean J Lab Med 2007; 27: 420-7.
47. Sung H, Park SJ, Woo YD, Choi BH, Kim MN. Evaluation of Seeplex RV detection kit for detecting rhinovirus, human metapneumovirus, and coronavirus. Korean J Lab Med 2008; 28: 109-17.
48. Kim S, Joo DH, Lee JB, Shim BS, Cheon IS, Jang JE, et al. Dual role of respiratory syncytial virus glycoprotein fragment as a mucosal immunogen and chemotactic adjuvant. PLoS One 2012; 7(2): e32226.
49. Erwin EA, Custis NJ, Satinover SM, Perzanowski MS, Woodfolk JA, Crane J, et al. Quantitative measurement of IgE antibodies to purified allergens using streptavidin linked to a high-capacity solid phase. J Allergy Clin Immunol 2005; 115(5): 1029-35.

50. Sander I, Kespohl S, Merget R, Goldscheid N, Degens PO, Bruning T, et al. A new method to bind allergens for the measurement of specific IgE antibodies. Int Arch Allergy Immunol 2005;136:39–44.
51. R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R-project.org/
52. Perkins NJ, Schisterman EF, Vexler A. Receiver operating characteristic curve inference from a sample with a limit of detection. Am J Epidemiol 2007; 165: 325–33.
53. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011; 12: 77.
54. Tan WC, Xiang X, Qiu D, Ng TP, Lam SF, Hegele RG. Epidemiology of respiratory viruses in patients hospitalized with near-fatal asthma, acute exacerbations of asthma, or chronic obstructive pulmonary disease. Am J Med 2003; 115: 272-7.
55. Thumerelle C, Deschildre A, Bouquillon C, Santos C, Sardet A, Scalbert M, et al. Role of viruses and atypical bacteria in exacerbations of asthma in hospitalized children: a prospective study in the Nord-Pas de Calais region (France). Pediatr Pulmonol 2003; 35: 75-82.
56. Khetsuriani N, Kazerouni NN, Erdman DD, Lu X, Redd SC, Anderson LJ, et al. Prevalence of viral respiratory tract infections in children with asthma. J Allergy Clin Immunol 2007; 119: 314-21.
57. Beasley R, Coleman ED, Hermon Y, Holst PE, O'Donnell TV, Tobias M. Viral respiratory tract infection and exacerbations of asthma in adult patients. Thorax 1988; 43: 679-83.
58. Kim YJ. Distribution of respiratory viruses of severe lower respiratory tract infections in infants and children. Public Health Weekly Report, KCDC 2011; 4(40): 725-9.
59. Korea Center for Disease Control and Prevention (KCDC). The Korea Influenza and Respiratory Surveillance System (KINRESS) Weekly

Surveillance Reports 2008-2013. Available from: http://www.cdc.go.kr/CDC/.
60. Hart RJC. An outbreak of respiratory syncytial virus infection in an old people's home. J Infect 1984; 8: 259-61.
61. Sorvillo FJ, Huie SF, Strassburg MA, Butsumyo A, Shandera WX, Fannin SL. An outbreak of respiratory syncytial virus pneumonia in a nursing home for the elderly. J Infect 1984; 9(3): 252-6.
62. Vikerfors T, Grandien M, Olcen P. Respiratory syncytial virus infections in adults. Am Rev Respir Dis 1987; 136(3): 561-4.
63. Fleming DM, Cross KW. Respiratory syncytial virus or influenza? Lancet 1993; 342(8886-8887): 1507-10.
64. Nicholson KG. Impact of influenza and respiratory syncytial virus on mortality in England and Wales from January 1975 to December 1990. Epidemiol Infect 1996; 116(1): 51-63.
65. Neuzil KM, Maynard C, Griffin MR, Heagerty P. Winter respiratory viruses and health care use: a population-based study in the northwest United States. Clin Infect Dis 2003; 37(2): 201-7.
66. Griffin MR, Coffey CS, Neuzil KM, Mitchel EF Jr, Wright PF, Edwards KM. Winter viruses: influenza- and respiratory syncytial virus-related morbidity in chronic lung disease. Arch Intern Med 2002; 162(11): 1229-36.
67. Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003; 289(2): 179-86.
68. Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010; 375(9725): 1545-55.
69. Nair H, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. Lancet 2011; 378(9807): 1917-30.

70. Gaunt ER, Harvala H, McIntyre C, Templeton KE, Simmonds P. Disease burden of the most commonly detected respiratory viruses in hospitalized patients calculated using the disability adjusted life year (DALY) model. J Clin Virol 2011; 52(3): 215-21.
71. Lysholm F, Wetterbom A, Lindau C, Darban H, Bjerkner A, Fahlander K, et al. Characterization of the viral microbiome in patients with severe lower respiratory tract infections, using metagenomic sequencing. PLoS One 2012; 7(2): e30875.
72. Karlsson EA, Beck MA. The burden of obesity on infectious disease. Exper Biol Med 2010; 235: 1412–24.
73. Falagas ME, Athanasoulia AP, Peppas G, Karageorgopoulos DE. Effect of body mass index on the outcome of infections: a systematic review. Obes Rev 2009; 10: 280–9.
74. Zimmerman RK, Rinaldo CR, Nowalk MP, Gk B, Thompson MG, Moehling KK, et al. Influenza and other respiratory virus infections in outpatients with medically attended acute respiratory infection during the 2011-12 influenza season. Influenza Other Respir Viruses 2014; 8(4): 397-405.
75. Sly PD, Kusel M, Holt PG. Do early-life viral infections cause asthma? J Allergy Clin Immunol 2010; 125(6): 1202-5.
76. Backman K, Piippo-Savolainen E, Ollikainen H, Koskela H, Korppi M. Adults face increased asthma risk after infant RSV bronchiolitis and reduced respiratory health-related quality of life after RSV pneumonia. Acta Paediatr 2014; 103(8): 850-5.
77. Backman K, Piippo-Savolainen E, Ollikainen H, Koskela H, Korppi M. Irreversible airway obstruction in adulthood after bronchiolitis in infancy: evidence from a 30-year follow-up study. Respir Med 2014; 108(1): 218-23.
78. Krishnamoorthy N, Khare A, Oriss TB, Raundhal M, Morse C, Yarlagadda M, et al. Early infection with respiratory syncytial virus impairs regulatory T cell function and increases susceptibility to allergic asthma. Nat Med. 2012; 18(10): 1525-30.

79. Luengo O, Cardona V. Component resolved diagnosis: when should it be used? Clin Transl Allergy 2014; 4: 28.
80. Stringari G, Tripodi S, Caffarelli C, Dondi A, Asero R, Di Rienzo , et al. The effect of component-resolved diagnosis on specific immunotherapy prescription in children with hay fever. J Allergy Clin Immunol 2014; 134(1): 75-81.
81. van Ree R, Chapman MD, Ferreira F, Vieths S, Bryan D, Cromwell O. The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. Allergy 2008; 63(3): 310-26.
82. Roh EJ, Jin YM, Chung EH, Rheem I, Kim JK. The prevalence of respiratory viral infection in exacerbation of asthma in hospitalized children. Korean J Asthma Allergy Clin Immunol. 2007; 27: 241-7.
83. Yoo JY, Kim DG, Eom KS, Shin T, Park YB, Lee JY, et al. The role of respiratory viral infections in exacerbation of asthma and chronic obstructive pulmonary disease (COPD). Tuberc Respir Dis. 2005; 59: 497-503.
84. Cheung DS, Grayson MH. Role of viruses in the development of atopic disease in pediatric patients. Curr Allergy Asthma Rep 2012; 12(6): 613-20.
85. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet 2010; 376(9743): 826-34.
86. Lee YW, Choi SY, Lee EK, Sohn JH, Park JW, Hong CS. Cross-allergenicity of pollens from the *Compositae* family: *Artemisia vulgaris, Dendranthema grandiflorum,* and *Taraxacum officinale.* Ann Allergy Asthma Immunol 2007; 99(6): 526-33.
87. Becker Y. HIV-1 gp41 heptad repeat 2 (HR2) possesses an amino acid domain that resembles the allergen domain in *Aspergillus fumigatus Asp f1 protein*: review, hypothesis and implications. Virus Genes 2007; 34: 233-40.
88. Nahm DH, Park HS. Analysis of induced sputum for studying allergen-specific IgE antibodies in airway secretion from asthmatic patients. Clin

Exp Allergy 1998; 28(6): 686-93.
89. Domínguez Ortega J, León F, Martínez Alonso JC, Alonso Llamazares A, Roldán E, Robledo T, et al. Fluorocytometric analysis of induced sputum cells in an asthmatic population. J Investig Allergol Clin Immunol 2004; 14(2): 108-13.
90. Araújo L, Palmares C, Beltrão M, Pereira AM, J Fonseca, Moreira A, et al. Validation of total and specic IgE measurements in induced sputum. J Investig Allergol Clin Immunol 2013; 23(5): 330-6.
91. Cao Y, Gong W, Zhang H, Liu B, Li B, Wu X, et al. A comparison of serum and sputum inflammatory mediator profiles in patients with asthma and COPD. J Int Med Res 2012; 40(6): 2231-42.
92. Khan SH, Park SS, Sirajuddin IA, Grayson MH. Respiratory virus and asthma: the role of immunoglobulin E. Clin Ther 2008; 30 Spec No:1017-24.
93. Baba S, Kondo K, Toma-Hirano M, Kanaya K, Suzukawa K, Ushio M, et al. Local increase in IgE and class switch recombination to IgE in nasal polyps in chronic rhinosinusitis. Clin Exp Allergy 2014 ; 44(5): 701-12.
94. Meng C, Sha J, Li L, An L, Zhu X, Meng X, et al. The expression and significance of immunoglobulin free light chain in the patients with allergic rhinitis and nonallergic rhinitis. Am J Rhinol Allergy 2014; 28(4): 302-7.
95. Ghebre MA, Bafadhel M, Desai D, Cohen SE, Newbold P, Rapley L, et al. Biological clustering supports both "Dutch" and "British" hypotheses of asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol 2014 Aug 13. pii: S0091-6749(14)00951-8. [Epub ahead of print]
96. Bafadhel M, McCormick M, Saha S, McKenna S, Shelley M, Hargadon B, et al. Profiling of sputum inflammatory mediators in asthma and chronic obstructive pulmonary disease. Respiration 2012; 83(1): 36-44.
97. Rothers J, Halonen M, Stern DA, Lohman IC, Mobley S, Spangenberg A, et al. Adaptive cytokine production in early life differentially predicts total IgE levels and asthma through age 5 years. J Allergy Clin Immunol 2011; 128(2): 397-402.
98. Crestani E, Lohman IC, Guerra S, Wright AL, Halonen M. Association

of IL-5 cytokine production and *in vivo* IgE levels in infants and parents. J Allergy Clin Immunol 2007; 120(4): 820-6.

99. Cheon IS, Shim BS, Park SM, Choi Y, Jang JE, Jung DI, et al. Development of safe and effective RSV vaccine by modified CD4 epitope in G protein core fragment (Gcf). PLoS One 2014; 9(4): e94269.
100. Bawage SS, Tiwari PM, Pillai S, Dennis V, Singh SR. Recent advances in diagnosis, prevention, and treatment of human respiratory syncytial virus. Adv Virol 2013; 2013: 595768.
101. Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, et al. A WAO-ARIA-GA²LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J 2013; 6(1): 17.
102. Normansell R, Walker S, Milan SJ, Walters EH, Nair P. Omalizumab for asthma in adults and children. Cochrane Database Syst Rev 2014; 1: CD003559.
103. Kumar A, Grayson MH. The role of viruses in the development and exacerbation of atopic disease. Ann Allergy Asthma Immunol 2009; 103(3): 181-6.

<ABSTRACT(IN KOREAN)>

성인 천식악화와 객담내 호흡기세포융합바이러스 특이 IgE 검출

<지도교수 박 중 원>

연세대학교 대학원 의학과

이 용 원

연구배경: 천식환자의 급성악화가 호흡기 바이러스 감염과 밀접하게 연관되어 있다고 믿어지고 있다. 바이러스 항원에 대한 감작 가능성을 고려하여, 몇몇 연구에서 마우스모델과 천식환자를 대상으로 호흡기합포체바이러스 (respiratory syn- cytial virus: RSV))에 대한 특이 면역글로불린 E (IgE)를 검출하고자 시도했다. 그러나 그러한 접근방법이 아직까지 완전히 확립되어 있지 않다.

연구재료 및 방법: 2년 동안, 호흡기 증상의 급성악화를 보인 한국 성인 천식환자군(60명)과 대조군(10명)의 하기도로부터 총 132개의 유도객담 검체를 수집하여 호흡기 바이러스 검출률, 종류, 계절적 변화 및 순차적 역동성을 연구하였다. 이들 검체를 가지고 12종의 호흡기 바이러스(adenovirus, rhino A virus, coronavirus OC43 및 229E/NL63, metapneumovirus, influenza A and B virus, parainfluenza viruses 1, 2, 3, 그리고 RSV A 및 B)에 대해 다중역전사중합효소연쇄반응(multiplex RT-PCR: mPCR)을 이용한 검출시험을 수행하였다. 그리고 50개의 객담검체를 선택하여 이들을 대상으로, RSV 조항원 및 RSV G단백질의 재조합 핵심조각(Gcf)에 대한 특이 IgE를 (유도객담 상층액 내에서) ImmunoCAP 시스템으로 검출하였고, ELISA를 이용하여 인터페론 감마, IL-5, 및 IL-13도 측정하였다.

연구결과: 132개의 유도객담 검체에 대한 mPCR 검사 결과 총 15건의 호흡기 바이러스 검출이 천식환자군 및 COPD 대조군(각각, 13 및 2건)에서 관찰되었다. 이들 중 RSV가 가장 빈번하게 검출된 종류였다(53%, 8/15). 천식환자 3명에서 다종검출 1건, 순차적 검출 2건이 관찰되었다. 천식환자군에서, 객담 상층액내 재조합 G 단백질 조각에 대한 CAP 특이 IgE (Gcf-sIgE)와 IL-5 수준은 mPCR에서 RSV가 검출된 적이 있는 군에서 더 높았다(각각, $p<0.05$). RSV-sIgE, Interferon-ɣ 및 IL13은 몇몇 연구대상자에서 검출되었으나 연구대상군 사이의 유의한 차이가 나타나지 않았다. CAP 특이 IgE를 측정한 천식환자군을 대상으로 ROC 분석을 시행한 결과, mPCR에서 RSV 검출을 예측하기 위한 Gcf-sIgE의 최적 좌표 기준치(the best coordinate cutoff)는 0.285 kU/L 였다(AUC 0.73, 95% CI 0.47-0.82; SE 81.3%, SP 65.4%, PPV 59.1%, NPV 85.0%).

결론: 이 연구에서, RSV는 성인 천식악화 환자의 객담검체에서 가장 높은 빈도로 검출된 바이러스 종류였다. 본 연구를 통해 통상적으로 사용되는 CAP 면역분석을 적용하여, RSV의 조항원 및 Gcf에 대한 특이 IgE 항체를 객담검체에서 검출하였다. mPCR 검사에서 RSV가 탐지된 적이 있는 성인 천식악화 환자군의 객담에서 Gcf 특이 IgE 및 IL-5 가 더 높게 측정된 것은, RSV 단백질에 대한 감작과 Th2 경로가 RSV와 관련된 성인 천식악화에 대해 역할을 가지고 있을 가능성을 시사한다.

핵심되는 말 : 성인 천식, 악화, 객담, 호흡기세포융합바이러스(RSV), 면역글로불린 E (IgE)